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Effect of vitamin E treatment on tissue fatty acids and cholesterol content in experimental diabetes

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The effects of 8 months of a pharmacological dose of vitamin E were studied on cholesterol content and fatty acid distribution in streptozotocin-diabetic rats under a controlled diet. Diabetes induced a decrease of monounsaturated fatty acids and particularly palmitoleic acid in all studied tissues: liver, aorta, plasma ($P < 0.01$, $P < 0.05$, $P < 0.05$, respectively). Vitamin E corrected partially Δ9 desaturase activity and consequently enhanced monounsaturated acids in all tissues. Concerning fatty acids of the n-6 series, diabetes induced an increase of C18:3 n-6 and C20:4 n-6 in liver associated with an overcorrection of Δ6 and Δ5 desaturase activities. Vitamin E normalized Δ6 activity in liver, and in aorta increased significantly C20:3 n-6 and C20:4 n-6 ($P < 0.005$, $P < 0.005$), which are fatty acid precursors of PGE1 and PGI2, prostaglandins protective for vessels. Fatty acids of the n-3 series decreased in the liver except C22:6 n-3; vitamin E again prevented these changes and increased in the aorta C22:5 n-3 and C22:6 n-3, which are involved in the generation of PGI3 whose activity is additive to PGI2. In plasma, polyunsaturated fatty acid pattern modifications were very similar to those of liver. Vitamin E was also able to prevent the decrease of free and esterified cholesterol observed in diabetic liver. Moreover, an increase of cholesterol in diabetic aorta was also modulated by vitamin E. Vitamin E could protect the long chain of polyunsaturated fatty acids, thereby preserving the physical properties of membranes and the environment necessary for enzymatic activities involved in fatty acid and cholesterol metabolism. Further, vitamin E in the aorta, by maintaining a high percentage of fatty acid precursors of protective prostaglandins, could represent an interesting therapy in diabetes to prevent atheroma. (J. Nutr. Biochem. 6:319–326, 1995.)

Keywords: tissue fatty acids; tissue cholesterol; streptozotocin-induced; diabetic rats; vitamin E

Introduction

Diabetes mellitus induces alterations of fatty acid patterns in various tissues in animals[1,2] as in humans, but in the latter, smaller and sometimes contradictory changes have been observed.[3,4] Fatty acid changes could play a role in the dysregulation of thromboxane/prostacyclin balance favoring atherothrombotic disease.[5] In streptozotocin-induced diabetic rats without treatment, major and consistent changes were an increase of stearic and linoleic acids associated with a decrease of oleic and arachidonic acids.[2] These modifications were due mainly to a depressed activity of Δ9, Δ6, and Δ5 desaturases. Insulin treatment of diabetic rats for only 2 days corrected and even overcorrected Δ9 and Δ6 desaturase activities.[2]

In our laboratory, we have shown, in streptozotocin-induced diabetic rats mildly balanced by insulin and fed 10 weeks on a diet containing 30% calories in lipids, a decrease in serum phospholipid fatty acids of the n-3 family (eicosapentaenoic and docosapentaenoic acids) and an increase of stearic acid associated with a decrease of monounsaturated acids (oleic and palmitoleic acids).[6] Very similar modifications in serum phospholipid fatty acids have been reported in insulin-dependent diabetic women.[7] These alterations in rats, as in diabetic patients, in part stem from decreased activities of Δ9 and Δ5 desaturases provoked by an insufficient metabolic control, but also perhaps by an oxidative status dysregulation inducing the loss of very long chain polyunsaturated fatty acids. Indeed, diabetes has been

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associated with an increase in the formation of free radicals[8] and an alteration of defenses against an oxidative stress.[9,10] In a previous work we have shown that vitamin E treatment at a high dose in diabetic rats mildly balanced by insulin could correct plasma fatty acids and decrease plasma oxidation markers,[6] while a moderate vitamin E supplement in insulin untreated streptozotocin-diabetic rats was ineffective in correcting the plasma fatty acid profile.[11]

This present work was undertaken to investigate whether a high dosage vitamin E treatment could maintain fatty acid composition after a long period of diabetes in tissues of streptozotocin-induced diabetic rats treated by insulin and submitted to a controlled diet. The streptozotocin diabetic rats treated by insulin for a time representing a long period in the life of a rat could be an attractive model to approach the changes in fatty acid content of inaccessible tissues induced by long-term diabetes in human patients. Indeed, plasma and tissue fatty acid composition might be maintained in rats as in humans by very similar mechanisms.[12]

Materials and methods

Animals and treatment

Forty male Sprague–Dawley rats with an initial body weight of 380 to 470 g were divided into three groups: C control (12 animals), D diabetic (16 animals), and DE diabetic vitamin E supplemented (16 animals). Diabetes was induced by IV injection of streptozotocin (30 mg/kg) dissolved in sodium citrate buffer, pH 4.5; control rats in group C were injected with buffer alone. Glycemia was determined in all animals 1 week after induction of diabetes. Only rats in groups D and DE with glycemia > 2.5 g/L 1 week after streptozotocin injection were considered diabetic and received 3.2 U of ultra-slow insulin subcutaneously 4×/week (Novo, Boulogne, France). All animals received a purified diet.[6] Briefly, it consisted of: proteins 15%, carbohydrates 52%, lipids 33% of calories, with a P/S ratio of 1. The vitamin mixture of the purified diet supplied 8 mg/100 g of vitamin E; in addition, in group DE, the purified diet was supplemented with tocopheryl acetate 115 mg/100 g (Sigma, St Louis, MO). Body weight was recorded each week. Urine samples were checked for ketone bodies and proteins with test strips (Rahignost Behring, Marburg, Germany).

All animals were housed and used in compliance with the National Institutes of Health and Medical Research policy on animal care and use, which is similar to the policy expressed by the NRC (1985) in its *Guide for the Care and Use of Laboratory Animals*.

Tissue preparations

Plasma. After 32 weeks of diabetes and a fast of 12 hr, blood from each rat was taken from the jugular vein under ether anesthesia. Blood was collected on an anticoagulant solution: sodium citrate 3.8% with pH adjusted to 7.4 with citric acid (1 vol anticoagulant/9 vol blood). Plasma samples of citrated blood obtained after centrifugation (1,500*g* for 15 min) were kept at −35°C until analysis.

Liver and aorta. After blood collection, the rats were killed by IV pentobarbital injection. The aorta and liver were immediately collected. Livers were carefully washed with NaCl 0.9% and then lyophylized. Thoracic and abdominal aortae were carefully freed of surrounding adipose tissue, cut into small rings, and lyophylized. Dessicated tissues were kept at −35°C until analysis.

Lipid extraction and analysis

Plasma. After 32 weeks of diabetes, plasma in the three groups of rats was extracted by the method of Folch et al.[13] The lipid extracts were dessicated under nitrogen. Plasma phospholipids were precipitated by acetone (Lichrosolv, Merck, Darmstadt, Germany), dessicated under sodium sulfate,[14] and kept overnight at 5°C before centrifugation (3000*g* for 10 min). Then phospholipids and acetone-soluble neutral lipids were dessicated under nitrogen. The lipid extracts obtained were dissolved in a mix of toluene/methanol (2 vol/3 vol) and methylated with BF_3/CH_3OH reagent (Merck) according to the method of Morrison and Smith.[15] The fatty acid methyl esters were separated by GLC in an Intersmat Instrument 120 DFL using a FID detector and a DB 23 column (30 m × 0.53 mm) (JW Scientific, Folsom, CA). The oven temperature was 128°C at injection and was maintained for 10 min, then raised by 2°C/min for 6 min, and then further raised to 235°C (4°C/min). Injector and detector temperatures were 150 and 250°C, respectively. Helium was used as carrier gas under a pressure of 0.8 bars. Plasma triglycerides, total and free cholesterol, and phospholipids were determined by enzymatic kits (BioMérieux, Marcy l'Etoile, France). Vitamin E in plasma was determined by HPLC following the technique of Leclerc et al.[16] with some modifications reported elsewhere.[6]

Liver and aorta lipids. Lyophilized tissues were homogenized in an Ultra-Turrax Instrument (Polytron Co., Luzern Switzerland) and a precise quantity of each homogenate (20 to 50 mg) was weighed. Liver and aorta lipids were extracted following the technique of Folch in chloroform/methanol 2/1 vol/vol.[13] Neutral lipids and phospholipids were separated, and the fatty acid methyl esters were obtained and analyzed as described earlier. To determine liver vitamin E, liver lipids were extracted after saponification following the technique reported by Rittenmaier with some modifications.[17] Briefly, 50 mg of liver was saponified 30 min at 100°C with 0.5 ml of a mix containing: methanol 0.19 ml, distilled water 0.31 ml, potassium hydroxide 0.31 g, and ascorbic acid 0.015 g. After cooling, 0.5 ml of distilled water was added, and lipids were extracted by 1 ml of a mix of heptane/toluene 1/1 (vol/vol) and dessicated under nitrogen before analysis by HPLC. Vitamin E in aorta was determined as reported before.[16] Free and total cholesterol were determined in aorta and liver following the technique of Omodeo-Sale.[18]

Statistical analysis

Unpaired Student's *t*-test preceded by ANOVA analysis was used to evaluate the significance of differences.

Results

Biological and plasma biochemical parameters in the three groups of rats—control (C), diabetic (D), and supplemented vitamin E diabetic (DE)—are shown in *Table 1*. After 32 weeks of diabetes, the weight of rats in group C was increased by 100% and only by 27% in group D, but in group DE the enhancement reached 43% and was significantly increased relative to group D ($P < 0.05$). Cataract development was inhibited by vitamin E treatment in diabetic rats as already observed.[6] Glycemia evaluated in the final period of insulin action (36 hr after injection) was significantly increased in the two diabetic groups compared with group C ($P < 0.001$). Plasma fructosamine was identical in the two diabetic groups. No significant changes in plasma lipids were observed in the three groups of rats.

Table 1 Clinical parameters and plasma biological parameters in control (C), and vitamin E treated or untreated (DE, D) streptozotocin-induced diabetic rats fed on controlled diet for 32 weeks

	C	D	DE
n =	12	11	14
Body weight gain (kg)	0.410 ± 0.023	0.118 ± 0.011*	0.205 ± 0.025†‡
Cataract incidence (%)	0	54.5	7.1
Glucose (mmol/L)	8.3 ± 0.3	20.3 ± 1.5*	21.9 ± 1.8†
Fructosamine (μmol/L)	166.8 ± 12.0	314.9 ± 16.6*	339.8 ± 26.0†
Free cholesterol (mmol/L)	0.59 ± 0.04	0.77 ± 0.08	0.77 ± 0.06
Cholesterol ester as fraction of total cholesterol	0.79	0.79	0.78
Total cholesterol (mmol/L)	2.90 ± 0.16	3.58 ± 0.35	3.42 ± 0.19
Phospholipids (mmol/L)	2.14 ± 0.12	2.47 ± 0.22	2.52 ± 0.14
Triglycerides (mmol/L)	3.01 ± 0.27	3.06 ± 0.45	3.97 ± 0.44

Results are mean ± SE for 11–14 experiments. C vs. D, $^*P < 0.001$; C vs. DE, $^\dagger P < 0.001$; D vs. DE, $^\ddagger P < 0.05$.

Vitamin E tissue concentrations are reported in *Table 2*. A significant increase of vitamin E in liver of diabetic rats was observed compared with control ($P < 0.05$), but vitamin E treatment in diabetic rats gave a 4 fold increase in vitamin E content in liver as compared with unsupplemented diabetic rats and a 5 fold increase compared with control rats. Vitamin E was also significantly augmented in other tissues in group DE compared with group C and significantly in aorta and plasma when compared with group D ($P < 0.05$).

Total, free, and esterified cholesterol levels in the liver and aorta of rats are shown in *Figure 1*. A large decrease in free and esterified cholesterol concentrations in liver of group D relative to group C was observed ($P < 0.001$, $P < 0.01$, respectively). Total and ester cholesterol in liver were completely normalized by vitamin E treatment in diabetic rats. In aorta, diabetes induced a significant increase of free cholesterol ($P < 0.05$) as compared with group C, and vitamin E supplement in diabetic rats normalized this increase.

The changes in fatty acid composition in liver and aorta of the three groups are reported in *Tables 3* and *4*, respectively. Concerning saturated acids, diabetes induced a significant decrease of palmitic acid (C16:0) in liver neutral lipids (NL) ($P < 0.05$) and in liver phospholipids ($P < 0.001$). In contrast, a significant elevation of stearic acid C18:0 was observed in the same fractions (NL $P < 0.05$, PL $P < 0.01$). In aorta lipids, only an increase of C18:0 in group DE relative to the other groups was noted ($P < 0.01$).

Monounsaturated fatty acids derived from C16:0 by the action of Δ9 desaturase, palmitoleic acid (C16:1 n-7), and vaccenic acid (C18:1 n-7) showed a significant decrease in diabetic liver NL ($P < 0.01$ and $P < 0.001$, respectively) as in diabetic aorta lipids ($P < 0.05$, $P < 0.01$) compared with the same tissues in group C. Vitamin E treatment in diabetic rats reduced the loss of these compounds in the two tissues, but an additional and significant decrease of oleic acid (C18:1 n-9) appeared in aorta of group DE as compared with groups C and D ($P < 0.01$).

Concerning polyunsaturated fatty acids, diabetes induced modifications in fatty acid distribution of the n-6 series. A significant increase of linoleic acid (C18:2 n-6) ($P < 0.01$) was noted. Linolenic acid (C18:3 n-6) derived from linoleic acid by the action of Δ6 desaturase was also significantly enhanced ($P < 0.05$) in liver PL of group D relative to group C. A significant increase of arachidonic acid (C20:4 n-6) ($P < 0.05$) was also observed in liver NL of group D relative to group C. Vitamin E treatment in diabetic rats cancelled these changes, except for linoleic acid, and in contrast induced a decrease of C20:4 n-6 ($P < 0.05$) in liver PL compared with group C.

In aorta, C18:2 n-6 and C18:3 n-6 were enhanced in group D compared with group C; however, the increase did not reach significance. These fatty acids were not modified in group DE, and a consistent rise of dihomo γ linolenic acid (C20:3 n-6) and C20:4 n-6 was noted in group DE relative to group D ($P < 0.05$) and C ($P < 0.005$).

The n-3 series in contrast to the n-6 series fatty acids were decreased in liver NL of group D compared with group C except for docosahexaenoic acid (C22:6 n-3), which in-

Table 2 Vitamin E in liver, aorta and plasma in control (C), diabetic (D), and vitamin E treated diabetic rats (DE), fed on controlled diet for 32 weeks

	Vitamin E		
Groups	C	D	DE
(*n*)	12	11	14
LIVER (μmol/g of dry tissue)	1.07 ± 0.09	1.39 ± 0.12*	5.44 ± 0.91†§
AORTA (nmol/g of dry tissue)	11.6 ± 2.3	9.3 ± 2.3	25.5 ± 4.6‡
PLASMA (μmol/L)	29 ± 3	39 ± 6	67 ± 8†‡

Results are mean ± SE for 11–14 experiments. Legend: C vs. D, $^*P < 0.05$; C vs. DE, $^\dagger P < 0.001$; D vs. DE, $^\ddagger P < 0.05$; $^\S P < 0.001$.

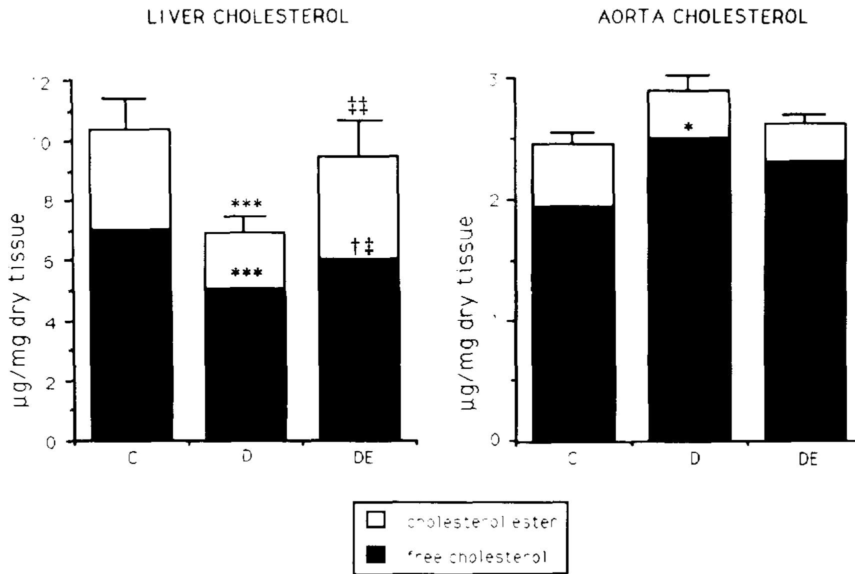

Figure 1 Cholesterol content in liver and aorta, in control (C) and vitamin E, treated or not (DE, D) streptozotocin-induced diabetic rats fed on a controlled diet for 32 weeks. Results are mean (±SE) for 11–14 experiments. Significance was, for total cholesterol: liver ***D vs. C, $P < 0.001$; ‡‡D vs. DE, $P < 0.01$, for free cholesterol: liver ***D vs. C, $P < 0.001$; †DE vs. C, $P < 0.05$; ‡DE vs. D, $P < 0.05$, and for aorta: *D vs. C, $P < 0.05$.

creased significantly ($P < 0.05$). Eicosapentenoic acid (C20:5 n-3) and docosapentaenoic acid (C22:5 n-3) were also significantly decreased ($P < 0.001$) in liver PL of group D as compared with group C. In liver NL, vitamin E treatment inhibited the loss of C20:5 n-3 and the accumulation of C22:6 n-3 but was able to limit changes only in liver PL.

In aorta lipids, no change was observed in fatty acids of the n-3 series in group D compared with group C, but in group DE a decrease of C18:3 n-3 for the benefit of C22:5 n-3 and C22:6 n-3 was noted, which were significantly increased ($P < 0.005$ and $P < 0.0005$, respectively).

A significant elevation of C20:3 n-9 ($P < 0.05$) in liver PL of group D compared with the other groups was also observed; in contrast, a significant decrease of this fatty acid was noted in liver NL of group DE relative to group D and group C.

Plasma fatty acid composition is reported in *Table 5*.

Table 3 Major fatty acid composition (%) and main fatty acid changes in liver, in control (C), and streptozotocin-induced vitamin E treated or untreated diabetic rats (DE, D) fed on controlled diet for 32 weeks

	Liver					
	Neutral lipids			Phospholipids		
Groups (*n*)	C 12	D 11	DE 14	C 12	D 11	DE 14
Saturated						
C16:0	20.6 ± 0.4	18.8 ± 0.6*	18.5 ± 0.5††	12.8 ± 0.2	10.4 ± 0.4***	10.3 ± 0.4†††
C18:0	6.4 ± 0.6	10.2 ± 1.4*	8.4 ± 0.7†	21.5 ± 0.3	23.8 ± 0.7**	23.7 ± 0.6††
Monounsaturated						
C16:1 n-7	2.9 ± 0.3	1.4 ± 0.4**	1.7 ± 0.4†	0.77 ± 0.1	0.53 ± 0.1	0.57 ± 0.1
C18:1 n-7	4.2 ± 0.2	2.8 ± 0.2***	3.6 ± 0.2‡	4.3 ± 0.1	3.6 ± 0.2**	4.3 ± 0.2‡
C18:1 n-9	22.4 ± 1.1	19.7 ± 1.3	22.3 ± 0.7	5.1 ± 0.1	5.3 ± 0.1	5.2 ± 0.12
Polyunsaturated						
n-6 Family						
C18:2 n-6	20.7 ± 0.7	19.2 ± 0.9	22.6 ± 0.4† ‡‡	16.9 ± 0.3	19.1 ± 0.5**	20.8 ± 0.6†††‡
C18:3 n-6	0.54 ± 0.04	0.53 ± 0.03	0.49 ± 0.04	0.12 ± 0.03	0.21 ± 0.03*	0.11 ± 0.03‡
C20:3 n-6	0.67 ± 0.03	1.1 ± 0.08***	0.92 ± 0.07††	1.8 ± 0.13	2.0 ± 0.02	2.1 ± 0.2
C20:4 n-6	10.5 ± 1.14	15.7 ± 1.2**	11.3 ± 0.7‡‡	24.8 ± 0.5	24.5 ± 1.2	23.2 ± 0.6†
n-3 Family						
C18:3 n-3	1.11 ± 0.08	0.68 ± 0.08**	0.89 ± 0.05†‡	0.15 ± 0.01	0.16 ± 0.01	0.14 ± 0.01
C20:5 n-3	0.28 ± 0.02	0.18 ± 0.01**	0.22 ± 0.02	0.17 ± 0.01	0.09 ± 0.01***	0.10 ± 0.01††
C22:5 n-3	0.71 ± 0.06	0.57 ± 0.03	0.62 ± 0.04	1.16 ± 0.07	0.72 ± 0.06***	0.90 ± 0.10†
C22:6 n-3	2.4 ± 0.2	3.2 ± 0.3*	2.6 ± 0.2	5.2 ± 0.2	5.2 ± 0.3	4.6 ± 0.2
n-9 Family						
C20:3 n-9	0.15 ± 0.01	0.19 ± 0.08	0.11 ± 0.01‡‡	0.19 ± 0.02	0.28 ± 0.03*	0.17 ± 0.02‡‡

Results are mean ± SE for 11–14 experiments. Legend: C vs. D, *$P < 0.05$; **$P < 0.1$; ***$P < 0.001$; C vs. DE, †$P < 0.05$; ††$P < 0.01$; †††$P < 0.001$; D vs. DE, ‡$P < 0.05$; ‡‡$P < 0.01$.

Table 4 Major fatty acid composition (%) and main fatty acid changes in aorta, in control (C), and streptozotocin-induced vitamin E treated or untreated diabetic rats (DE, D) fed on controlled diet for 32 weeks

	Aorta		
	Total lipids		
Groups (*n*)	C 12	D 11	DE 12
Saturated			
C16:0	19.7 ± 0.3	16.6 ± 0.4***	17.2 ± 0.3†††
C18:0	11.7 ± 0.5	12.6 ± 0.7	14.2 ± 0.5††
Monounsaturated			
C16:1 n-7	1.80 ± 0.2	0.95 ± 0.2*	1.06 ± 0.2†
C18:1 n-7	3.8 ± 0.1	3.4 ± 0.1**	3.2 ± 0.2†
C18:1 n-9	25.4 ± 1.1	25.2 ± 1.2	18.7 ± 1.6†† ‡‡
Polyunsaturated			
n-6 Family			
C18:2 n-6	14.8 ± 0.6	16.1 ± 0.7	13.8 ± 0.6‡
C18:3 n-6	0.14 ± 0.03	0.21 ± 0.02	0.11 ± 0.02‡‡
C20:3 n-6	0.44 ± 0.04	0.51 ± 0.04	0.62 ± 0.02†† ‡
C20:4 n-6	8.6 ± 0.7	9.5 ± 0.9	12.4 ± 1.0†† ‡
n-3 Family			
C18:3 n-3	0.53 ± 0.02	0.56 ± 0.04	0.44 ± 0.04‡
C20:5 n-3	0.14 ± 0.03	0.12 ± 0.01	0.12 ± 0.02
C22:5 n-3	0.31 ± 0.03	0.36 ± 0.03	0.45 ± 0.03†† ‡
C22:6 n-3	1.1 ± 0.08	1.3 ± 0.1	1.9 ± 0.1††† ‡
n-9 Family			
C20:3 n-9	0.14 ± 0.05	0.13 ± 0.04	0.14 ± 0.02

Results are mean ± SE for 11–14 experiments. Legend: C vs. D, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; C vs. DE, †$P < 0.05$; ††$P < 0.01$; †††$P < 0.001$; D vs. DE, ‡$P < 0.05$; ‡‡$P < 0.01$.

Plasma fatty acid changes in group D and DE compared with group C were less numerous and smaller than those observed in liver and aorta.

In plasma, monounsaturated fatty acids as in liver and aorta showed a significant decrease of C16:1 n-7 in all studied fractions in group D as compared with group C (plasma NL, $P < 0.05$; plasma PL, $P < 0.05$). Vitamin E treatment in diabetic rats inhibited the lowering of C16:1 n-7 in plasma. Moreover, C18:1 n-7 was diminished in plasma PL in group D as in group DE compared with group C.

Fatty acids of the n-6 series presented only one modification in plasma of the two diabetic groups, i.e., a significant elevation of C20:3 n-6 ($P < 0.05$ for both groups) compared with group C.

Alterations in the fatty acid profile of the n-3 series in plasma NL were very similar to those observed in liver NL. A notable diminution was observed for C18:3 n-3, C20:5 n-3, C22:5 n-3 ($P < 0.001$ for all groups). Vitamin E partially corrected these changes.

Discussion

Fatty acid composition in any tissue reflects both the activity of esterifying enzymes and the quantities of the different fatty acids available to the enzymes.[19] Many alterations in these processes have been reported in diabetes mellitus. Fatty acyl transferase and desaturase activities are altered in liver of streptozotocin-induced diabetic rats and could together explain alterations in fatty acid composition in diabetic animals.[20] A decrease of palmitoleic acid, oleic acid, and arachidonic acid associated with an increase of linoleic acid and docosahexaenoic acid were observed in microsomal liver in diabetic rats.[21] Changes in the fatty acid profile of diabetic rats have also been reported in various tissues and were almost always corrected or overcorrected by a relatively short treatment of insulin.[5]

In this work, fatty acid composition of tissues of diabetic rats were studied after a long period of disease and insulin treatment and under a controlled diet. We attempted to approach diabetic human conditions where a weak but persistent alteration of regulation processes involved in the maintenance of tissue fatty acid composition and oxidative status could be involved in the genesis of cardiovascular disease observed in this pathology.

In our conditions, we did not observe a complete correction of Δ9 desaturase in the diabetic group treated by insulin, because C16:1 n-7 was decreased in all studied tissues in group D compared with group C. In streptozotocin rats on a laboratory diet after 7 weeks of diabetes and some days of insulin treatment, an overcorrection of Δ9 desaturase has been reported.[5] Insulin, as well as thyroxin and epinephrine, produces an increase in the level of Δ9 desaturase; how this is related to dietary changes is not clear.[2] Besides, in group D we noted that the decrease in monounsaturated acids in liver, aorta, and plasma were observed essentially in the n-7 series and could depend in part on a decrease of C16:0. A diminution of C16:0 in microsomal phospholipid composition in streptozotocin-diabetic rats was already reported after only 14–21 days of diabetes and was corrected by only 2 days of insulin treatment.[21] The same observation was reported in aorta; a significant reduction of C16:0 level was noted in streptozotocin-diabetic rats after 7 weeks of diabetes and restored by insulin.[5] In this study, after 8 months of diabetes mildly balanced by insulin, a significant decrease of C16:0 persisted in aorta, liver, and plasma without however reaching significance in the latter. This decrease was accompanied by an increase of C18:0. In a previous work, this change was attributed in aorta to the triglyceride fraction of the fat deposit in that tissue.[5] In our conditions, fat deposits in aorta were very carefully removed under the microscope and the same changes were observed in liver and certainly indicated a real fatty acid change in the concerned tissues. This decrease of C16:0 might be due to an unchanged elongation of C16:0 versus C18:0, whereas the Δ9 desaturase activity was decreased in these tissues.

Concerning fatty acids derived from Δ6 desaturase activity, for C18:3 n-6 an overcorrection in diabetic rat liver was observed in our conditions because a significant augmentation of C18:3 n-6 was noted in liver PL. A preferential increase of C20:4 n-6 in liver NL rather than PL could also be associated with an overcorrection of Δ5 desaturase but might reflect an alteration of metabolic control in diabetic rats.[22]

In rats, a long-term diabetes, mildly balanced by insulin, induced a decrease in fatty acids of the n-3 series in liver

Table 5 Major fatty acid composition (%) and main fatty acid changes in plasma, in control (C), and streptozotocin-induced vitamin E treated or untreated diabetic rats (DE, D) fed on controlled diet for 32 weeks

	Plasma					
	Neutral lipids			Phospholipids		
Groups	C	D	DE	C	D	DE
(*n*)	12	11	14	11	11	13
Saturated						
C16:0	17.5 ± 0.2	16.8 ± 0.4	16.8 ± 0.3	18.8 ± 0.6	17.3 ± 0.7	16.7 ± 0.6†
C18:0	5.3 ± 0.3	7.0 ± 0.5**	6.3 ± 0.3†	25.8 ± 0.7	29.3 ± 1.1*	29.9 ± 1.0††
Monounsaturated						
C16:1 n-7	2.7 ± 0.3	1.5 ± 0.3*	1.7 ± 0.4	0.40 ± 0.09	0.19 ± 0.09	0.23 ± 0.05
C16:1 n-9	0.65 ± 0.08	0.56 ± 0.08	0.70 ± 0.04	0.13 ± 0.06	0.06 ± 0.03	0.09 ± 0.04
C18:1 n-7	26.8 ± 0.7	25.6 ± 0.6	25.6 ± 0.7	2.9 ± 0.2	2.3 ± 0.2	2.2 ± 0.1†
C18:1 n-9				6.0 ± 0.2	6.2 ± 0.2	5.9 ± 0.2
Polyunsaturated						
n-6 Family						
C18:2 n-6	23.8 ± 0.5	24.2 ± 0.8	24.5 ± 0.8	12.1 ± 0.6	13.3 ± 0.9	11.7 ± 0.7
C18:3 n-6	0.67 ± 0.04	0.70 ± 0.06	0.71 ± 0.06	0.04 ± 0.08	0.03 ± 0.05	0.06 ± 0.02
C20:3 n-6	0.69 ± 0.04	0.85 ± 0.07*	0.87 ± 0.06†	1.44 ± 0.1	1.64 ± 0.2	1.8 ± 0.2
C20:4 n-6	12.9 ± 0.6	14.6 ± 0.9	14.0 ± 1.1	18.8 ± 0.6	18.2 ± 0.6	19.4 ± 0.7
n-3 Family						
C18:3 n-3	1.06 ± 0.05	0.74 ± 0.05***	0.83 ± 0.07†	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
C20:5 n-3	0.52 ± 0.05	0.29 ± 0.02***	0.37 ± 0.02†‡	0.06 ± 0.01	0.05 ± 0.02	0.05 ± 0.01
C22:5 n-3	0.62 ± 0.1	0.41 ± 0.03***	0.53 ± 0.04‡	0.51 ± 0.05	0.36 ± 0.03*	0.36 ± 0.02††
C22:6 n-3	1.56 ± 0.08	1.6 ± 0.1	1.7 ± 0.08	4.0 ± 0.3	3.7 ± 0.2	4.0 ± 0.2
n-9 Family						
C20:3 n-9	0.58 ± 0.03	0.50 ± 0.03	0.58 ± 0.02	0.24 ± 0.06	0.11 ± 0.02	0.11 ± 0.02†

Results are mean ± SE for 11–14 experiments. C vs. D, **P* < 0.05; ***P* < 0.01; ****P* < 0.001; C vs. DE, †*P* < 0.05; ††*P* < 0.01; D vs. DE, ‡*P* < 0.05.

and plasma neutral lipids, especially in C18:3 n-3. In relative terms, an increase of fatty acids of the n-3 series in tissues of streptozotocin diabetic rats has often been noted.[2,5] However, in absolute terms, on measurement of the fatty acids of the total lipids in whole animal, all polyunsaturated (n-6 and n-3) acids are reported to be decreased in diabetes.[23] In diabetic rats, after a long-term treatment by insulin (our study), a notable loss of C18:3 n-3, C20:5 n-3, and C22:5 n-3 was observed in liver NL of the diabetic animal, but C22:6 n-3 paradoxically was significantly increased. These changes might be due to an overcorrection of Δ4 desaturase activity and Δ5 desaturase, which induced a large increase of C20:4 n-6 in the same fraction, but also to an insufficient metabolic control because an increased proportion of C22:6 n-3 in diabetic rat liver has also been observed before insulin treatment.[23] Diminutions of C20:5 n-3 and C22:5 n-3 were also observed in liver PL, without loss of C18:3 n-3; these relatively small modifications in the PL fraction compared with NL could result from an enhancement in liver enzyme activity involved in the formation of phospholipids containing highly polyunsaturated fatty acids.[20]

High dosage vitamin E treatment in diabetic rats enhanced significantly the vitamin E content in all studied tissues, especially the liver. Vitamin E supplementation in diabetic rats partially corrected changes in saturated and monounsaturated fatty acids and consequently Δ9 desaturase activity in liver as in other tissues, as already observed in plasma of streptozotocin-diabetic rats in our previous study.[6] However, in all studied tissues, vitamin E increased C16:1 n-7 and other monounsaturated acids that have been associated with considerable antioxidant properties.[24]

Concerning the fatty acids of the n-6 series, vitamin E restored the fatty acid profile in liver NL into one similar to the control group. Moreover, vitamin E prevented in PL the increase of C18:3 n-6 observed in the diabetic group without supplementation and therefore normalized Δ6 desaturase activities.

In aorta lipids in the diabetic group treated by vitamin E, the increase of C18:3 n-6, C20:3 n-6, and C20:4 n-6 compared with group C and D may have been associated with an overcorrection of Δ6 and Δ5 desaturase activities, but the increase of C20:4 n-6 could also have been due to phospholipase A2 activity normalization, reported to be lowered by diabetes and stimulated by insulin.[20] The enhancement of vitamin E in tissues could also ensure both a better protection of polyunsaturated fatty acids against oxidation processes and perhaps an increased activity of desaturases by a beneficial antioxidant effect on these enzymes.[25] It is important to note that the increase in aorta by vitamin E supplementation of C20:3 n-6 and C20:4 n-6 precursors, respectively, of PGE1[26] and PGI2 prostaglandins known as platelet aggregation inhibitors[27] and as potent vasodilators,[28] could be a very important effect of this vitamin in the prevention of vascular alterations associated with diabetes.

Moreover, vitamin E minimized the diminution of fatty acids of the n-3 series in liver and in plasma and enhanced in aorta C22:5 n-3 and C22:6 n-3, which could produce by retroconversion C20:5 n-3,[29] a precursor of PGI3, whose activity is additive to PGI2.[30] Vitamin E prevented also the

rise of C20:3 n-9 in liver PL, an increase that could signify a relative lack of essential fatty acids in diabetic rats and also produce an increase in peroxidation.[31]

Another protective effect of vitamin E on cholesterol in the liver of diabetic rats must be noted. Vitamin E treatment in diabetic rats increased and restored liver cholesterol content similar to the control group. At the same time, the cholesterol content in the aorta decreased in the diabetic group with vitamin E supplementation. These findings suggest a better reverse transport of cholesterol and/or an increase of cholesterol synthesis in liver associated with a large elevation of vitamin E content. Another explanation is that the liver is the vitamin E stockage organ and an increase of this vitamin in situ could decrease oxidation products such as oxysterols, known as potent inhibitors of cholesterol synthesis.[32] In contrast, these products could increase in nonsupplemented vitamin E diabetic rats, thereby inducing the lowered cholesterol content observed. A decrease of liver cholesterol synthesis in diabetic rats has been reported.[33] It is also interesting to note that vitamin E restored cholesterol esterification in diabetic liver. By maintenance of cholesterol content and fatty acid distribution in membranes, vitamin E protects properties of the membrane surrounding necessary for acyl transferase activity (ACAT) and cholesterol synthesis.[34] Vitamin E, in decreasing oxidative processes in diabetes, could act in maintaining both fatty acid distribution as well as cholesterol content in tissues.

Vitamin E supplementation recently has been associated with a lowered risk of cardiovascular disease independently of other risk factors.[35,36] In diabetes, where cardiovascular complications represent the major cause of mortality,[37] and where oxidative processes are dysregulated,[8] vitamin E supplementation could be an interesting adjunct therapy. Recently, vitamin E supplement in diabetes has been associated with a better glycemic control,[38] but in our work we have not observed a fructosamine reduction in vitamin E supplemented diabetic rats after 8 months of diabetes as compared with vitamin E untreated diabetic rats. In this study, we showed in streptozotocin-diabetic rats, after a long-term insulin treatment, persistent and significant tissue lipid alterations. Vitamin E supplementation in diabetic rats had several positive effects: (1) decreased cataract incidence, (2) total or partial restoration of liver and aorta fatty acid distribution in lipid fractions, (3) an increase in aorta fatty acid precursors of protective prostaglandins concerning atheroma development, (4) protection of monounsaturated and long chain polyunsaturated fatty acids in all studied tissues that could play a role in the maintenance of physical properties of membranes associated with enzyme activities, (5) modulation of cholesterol content in liver contributing to cholesterol homeostasis in tissues. Studies on vitamin E supplementation effects in human diabetic patients should be undertaken because of its lack of toxicity and the therapeutic perspectives that it could offer in this disease.

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